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Determining the Specificity and Sensitivity of Previously Identified Human, Gull, Ruminant, and Poultry Genetic Markers  
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Jessica Tague

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Signatures of Honors Thesis Committee

Mentor: Mark F. Frana MARK F. FRANA

Reader 1: Lauren R. Hill Lauren R. Hill

Reader 2: Barbara J. Pollock BARBARA J. POLLOCK

Director: [Signature] JAMES J. BUSS

Signature

Print

## Determining the Specificity and Sensitivity of Previously Identified Human, Gull, Ruminant, and Poultry Genetic Markers

### **Abstract:**

Fecal contamination of environmental waters is of major concern due to its potential to contaminate shellfish and recreational waters, posing a threat to human health. Microbial source tracking (MST) is an important methodology for detecting the source of fecal contamination by probing water samples for genetic markers uniquely associated with bacteria from a specific host. The level of performance of genetic markers is measured by testing their specificity and sensitivity which can vary due to geographic and environmental conditions. In this project four genetic markers were tested for their specificity (n=15, 17, or 18) and sensitivity by analyzing the products of PCR reactions containing DNA from known fecal sources and primers that target each specific marker. It was determined that the human primers, HF183, were 89.5% specific and 100% sensitive. Gull primers, Gull2, were 70.5% specific and 75% sensitive. Lastly, the poultry primers, LA35, were determined to be 100% specific and 50% sensitive. An analysis of ruminant primers, RumBac2, was inconclusive; further processing will be completed.

### **Introduction:**

The conservation of water sources for both recreational and commercial use is extremely important; any potential harm to the human population must be identified (Scott et. al 2002). The outbreak of disease has increased since the quality of water has

deteriorated (Araujo et. al 2014). Bacteria that colonize the intestines, known as enterics, can cause numerous human diseases, such as gastroenteritis when from an infected source. Such bacteria can become pathogenic when transmitted to immunocompromised humans through the fecal-oral route (Johnson 135). There are numerous ways fecal contamination can reach water ways, such as direct discharge of contaminated water, run off from pets and livestock, failing septic systems, waste water treatment plants, and wildlife (Ahmed et. al 2016, Boehm et. al 2013, Jofre et. al 2014,). Human fecal contamination in waterways is thought to pose the greatest risk to human health compared to contamination from non-human species. Currently, evidence regarding the risk to the general public after exposure to non-human fecal matter is lacking, however, fecal material from other sources may still present a risk (Layton et. al 2013).

Aside from human contamination, waterfowl species, such as seagull, have been shown to contribute to the contamination of beaches and coastal waterways. Gull feces can carry potentially pathogenic bacteria, therefore exposing humans to additional harmful sources of disease (Lu et al. 2008, Sinigalliano 2013). The targeted fecal bacteria in gulls, *Catellibococcus marimammalium*, can be involved in the transmission of disease through aquatic animals (Lu et. al 2008). This species of bacteria was originally isolated from a porpoise, inferring that it has since traveled from avian species to aquatic species. According to Rabbia et. al, gulls have recently been identified to carry antibiotic resistant strains of *Escherichia coli* as well (2016). Their yearlong presence around coastal waterways, such as the Eastern Shore of Maryland and the coast of Delaware, provides a strong correlation to fecal indicator bacteria in waterways (Ryu et al. 2012). Poultry litter has also been known to carry infectious agents that can be transmitted when

carried through water routes. The presence of species such as *Salmonella enterica* and *Campylobacter jejuni* in poultry litter raises public concern (Nayak et. al 2015). *Escherichia coli* O157:H7, a toxic strain of *E. coli* has been found in a notable percentage of ruminant waste. This strain is shed in the waste, which is then used as manure. This species survives for long periods of time in the environment, and contaminated manure is added to fields for crops and spread through runoff (Solomon 2002).

Environmental waters are routinely monitored for the presence and quantity of fecal indicator bacteria (FIB) in order to determine the extent of fecal contamination. Fecal indicator bacteria are present in numbers possibly as high as  $10^{13}$  (Krause et. al 1) in the gastrointestinal tract of humans and animals and generally absent from an uncontaminated environment (Yost et. al 67). FIB have distinct characteristics such as a low survival time in the environment (Ahmed et. al 2016). *Escherichia coli* is a FIB species found ubiquitously in humans and other animals (Mattioli et. al 2017). *E. coli* is shed in high quantity per gram of feces, indicating contamination when found in water samples. *Enterococci* spp. is another FIB used to detect recent contamination (Mattioli et. al 2017). Particular species, such as *E. faecium* and *E. faecalis*, raise additional concern because of their antibiotic resistance (Yost et. al 71). Enterococci presence in water samples can be quantified in 24 hours using Enterolert ® or *E. coli* can be quantified using Colilert. Both tests quantify the presence of FIB in a given water sample. Although *E. coli* and *Enterococci* spp. indicate contamination, their presence does not indicate the source. When FIB are in high density, it is important to identify the main source of contamination.

Microbial source tracking (MST) incorporates numerous techniques used to distinguish the origin of fecal contamination. Source tracking relies on detecting the differences in bacterial properties resulting in different intestinal environments among host species. A genotypic method of microbial source tracking involves unique genetic sequences that are associated with specific strains of bacteria. Due to the concern of public safety, there should be a strong correlation between a chosen MST species of bacteria and FIB (Ahmed et. al 2016). There should be a high concentration of targeted fecal bacteria in fecal material and it should be evenly distributed throughout the fecal matter (Ahmed et. al 2016). The genus *Bacteroides* has previously been identified as a useful candidate for MST such that this genus has been identified as fecal bacteria of numerous host species (Ahmed et. al 2016, Riedel 2014). This genus is present in large numbers within the human (Scott et. al) and ruminant species gut microbiomes (Mieszkin 2009) and has a low culturability when exposed to the environment (Haugland et al 2010, Mieszkin 2009). The species *Catellibacterium marimammalium* has been identified as a gull fecal bacterium useful for MST studies because it is found in the gastrointestinal tract of waterfowl (Sinigalliano et al. 2013). According to Weidhaas et. al, only one genus of poultry fecal bacteria has been verified as useful when trying to determine the source of poultry contamination (2010).

Sources of interest within this study have unique gastrointestinal microbiomes that can be targeted with genetic markers. Genetic markers are sequences of DNA that can detect a specific location in the genome of a given bacterial species associated with a given host. The markers we will be using have been previously developed to recognize the 16S region of the ribosomal RNA of specific bacteria in each subsequent species host

gut microbiome. This specific region of bacterial rRNA is highly conserved, allowing for recognizable genetic diversity among hosts (Chakravorty 2007). It is important to study a conserved region of the genome because a high mutation rate would lead to inaccuracy when detecting specific fecal bacteria.

In order to confidently use a genetic marker for microbial source tracking in a specific geographic region, the accuracy of each genetic marker should be determined prior to the study (Green et. al 2014). By determining the specificity, we can verify the accuracy of our chosen genetic markers. Our chosen genetic markers should not cross react with multiple species leading to false positive detection. We determined the sensitivity of our chosen genetic markers, ensuring that any contamination of the species of interest will be identified in all water samples. Primers designed to detect the 16S regions of fecal bacteria located in the gut microbiomes of Humans (Haugland et. al 2010), Gulls (Lu et. al 2008), Poultry (Weidhaas et. al 2010), and Ruminants (Mieszkin et. al 2010) have been previously developed and tested for their ability to detect the source of interest. Primers used in this study have had variable specificity and sensitivity in specific geographic regions in the United States. In a previous study, the Human primers, HF183 (Haugland et. al 2010), was determined to be 96% specific and 75% sensitive (Layton et. al 2013). The Gull primers, Gull2 (Lu et. al 2008), was determined to be 71% sensitive and 100% specific from samples collected in Florida, West Virginia, Ohio, Georgia, and Ontario, Canada. According to a study by Weidhaas et. al, the poultry primers, LA35 was found to be 80% sensitive and 93% specific against samples collected in Florida, Georgia, Minnesota, and Utah. The ruminant primers, RumBac2 (Mieszkin et. al 2010), was found to be 97% sensitive and 100% specific within their

sample size from samples in Normandy and Brittany, France. By determining the specificity and sensitivity of our chosen primers on the Eastern Shore of Maryland and Delaware, we can confidently assess water samples from this region for the source of contamination.

## **Materials and Methods:**

### **Sample Collection**

Determining the sensitivity and specificity of each primer set requires scat samples from known sources living in the geographic region of interest, including those of focus for the study. Eighteen samples were either donated or collected from the various locations along the Eastern Shore of Maryland and Delaware (Table 1). Samples were donated to the microbial source tracking lab from Michael Bott of the Delaware Department of Natural Resources and Environmental Control, Allison Marine of the Wicomico County Health Department, and Dr. David Pollock. Sampling sites include but are not limited to the Wicomico County Landfill, Seaford, Lewes, and Rehoboth Waste Water Treatment Plants, Ocean City, MD, Somerset County, MD, and Sussex County, DE. Samples were stored at -80°C in the microbial source tracking lab until further processing.

### **DNA Extractions**

A small quantity of each fecal sample (Table 1.), ranging from 0.10- 0.30 g, was processed using a PowerSoil DNA isolation kit (MoBio laboratories, Inc., Carlsbad, CA) according to manufacturer's instructions. The final concentration of each DNA extraction was measured using a Thermo Scientific Nanodrop 1000, and each final concentration of

DNA was diluted with water to 2 µg/µl. The duck sample had a final concentration of 1.4 µg/ µl, perhaps due to the low water content of the original fecal sample.

Table 1. Location of donated and collected samples within Maryland and Delaware

<b>Human (3)*</b>	Seaford, DE Waste Water Treatment plant Lewes, DE Waste Water Treatment plant Rehoboth, DE Waste Water Treatment plant
<b>Gull (8)</b>	Ocean City, MD (5) Wicomico County, MD landfill (3)
<b>Poultry (7)</b>	Somerset County, MD Dover, DE Wicomico County, MD
<b>Cow (2)</b>	Sharpsburg, MD
<b>Dog</b>	Salisbury, MD
<b>Rabbit</b>	Salisbury, MD (domestic)
<b>Deer, Muskrat, Morning Dove, Raccoon (2), Dairy Goat, Duck</b>	Donated by Delaware Department of Natural Resources and Environmental Control

\*() = number of samples

### Standard Concentration Analysis

The human, gull, and ruminant positive control sequences were synthesized by Integrated DNA Technologies, Inc., Coralville, IA, and the poultry litter known sequence was provided by Jennifer Weidhaas, from the University of West Virginia. Positive control samples, known as standards, originally at a concentration of  $1 \times 10^9$  copies/µl were diluted to 10 copies/µl using a 1:10 serial dilution. In order to determine the correct concentration of standard to be used in remaining gels, each standard serial dilution was run in PCR against the matching genetic markers. It was determined that  $1 \times 10^4$  copies/µl was a sufficient concentration of standard resulting in visually appealing amplification.

**Human/Gull/Ruminant Marker Protocol**

The human primers, HF183/BacR287 was originally described by Haugland et. al 2010. The forward and reverse primers have a concentration of 800 nM. Lu et. al described the gull primer, Gull2 in 2008. Both the forward and reverse primers were used at 1000 nM. The primers used to detect Ruminant contamination, RumBac2, was originally described by Mieszkin et. al 2010. The original concentration of both primers was 1000 nM. Polymerase chain reaction (PCR) conditions include 50°C for 1 minute, 25°C for 10 minutes, followed by 40 cycles of 95°C 15 seconds and 60°C for 1 minute.

**Poultry Marker Protocol**

The poultry primer, LA35, was originally described by Weidhaas and Lipscomb in 2013. The PCR conditions used were 15 minutes at 95°C, followed by 40 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. The concentration of the LA35 primers is 1000 nM.

**Endpoint Polymerase Chain Reaction (PCR)**

PCR reactions took place in GE Healthcare Life Sciences Illustra Ruretaq Read-to-go PCR beads. Each PCR tube contained the reaction bead, 2 µl of the forward and reverse primer (human, gull, and ruminant) or 2.5 µl of each primer (poultry), 5 µl of DNA sample, and water to a final reaction volume of 25 µl. The positive control included 5ul of  $1 \times 10^4$  copies/µl standard DNA to be amplified by the primer set. Negative controls contained 5 µl of water instead of DNA.

### **Agarose Gel Electrophoresis**

Agarose gel electrophoresis was used to process the PCR product. 1X speed buffer was made using 0.1 M NaOH, boric acid to a pH of 8.0 and  $2.5 \times 10^{-4}$  mg/ml ethidium bromide (stock: 10 mg/ml) brought up to 1 L with water. Contents are stored in dark at room temperature. The correct fragment length was confirmed using an exACTGene Low Range Plus DNA Ladder from Fisher Scientific International Inc.

### **Human Specificity and Sensitivity**

In order to determine the specificity of the HF183 primers, 18 samples collected along the Eastern Shore of Maryland and Delaware were used. Sensitivity of HF183 was evaluated using three influent water samples from three waste water treatment plants in DE: Lewes, Seaford, and Rehoboth. A sequence 167 base pairs in length is amplified by the HF183 primer set.

### **Gull Specificity and Sensitivity**

Gull2 was tested against the 18 DNA extractions in order to determine its specificity using species found on the Eastern Shore of Maryland and Delaware. Gull fecal samples were collected from the Wicomico landfill and from the beach in Ocean City, MD in order to determine the sensitivity of the Gull2 primer. The DNA was extracted and tested against the Gull2 primers in order to determine if this primer set continuously identifies gull fecal bacteria. Positive amplification is a 512 base pair fragment of DNA.

### **Poultry Specificity and Sensitivity**

The LA35 molecular marker has been previously identified to have high specificity and sensitivity when identifying poultry litter. The 18 DNA extractions were amplified against the LA35 genetic marker using PCR, and then visualized using gel electrophoresis. Of the 18 samples, three of them were from poultry houses in Wicomico County, MD, Dover, DE, and Somerset County, MD, in order to quantify the sensitivity of LA35, in total 7 chicken samples were amplified, and visualized using gel electrophoresis. Positive amplification results in a 571 bp fragment.

### **Ruminant Specificity and Sensitivity**

The RumBac2 primers described by Mieszkin et. al 2010 amplifies a fragment roughly 100 base pairs in length. In order to determine its specificity in regards to species found on the Eastern Shore, 18 DNA extractions were used.

### **Analysis**

In order to calculate the specificity of a primer set, the equation  $\frac{d}{b+d} \times 100$ , where b is the number of false positives (non-target hosts indicate positive amplification), and d is the number of true negatives (non-target hosts are negative for amplification) (Ahmed et. al 2016). To calculate the sensitivity of each genetic marker,  $\frac{a}{a+c} \times 100$  was used, where a is the number of true positive results (positive amplification when present in the sample) and c is the number of false negative results (negative amplification when present in the sample).

**Results:****Human**

According to Haugland et. al, the HF183 primer set amplifies a 126 basepair fragment. Of the 18 non-human samples, there was possible cross-reactivity with rabbit, dairy goat, and one of the three original chicken samples (2010). The band length for the rabbit amplification was located at the same base pair length as the positive control poultry standard (Figure 3, lane 2). Possible cross reactivity was found in dairy goat and chicken as well, but the band was slightly higher than the positive control (Figure 2, lanes 2-3). In order to validate positive cross-reactivity of rabbit, chicken, and dairy goat, duplicate PCR reactions were tested, in addition to three WWTP samples. (Figure 4, Figure 5) When compared to the HF183 standard and WWTP amplified fragment lengths, the faint bands originally observed in dairy goat and rabbit, appeared noticeably higher (Figure 4, lanes 2-6). The HF183 primers amplified one original chicken sample at the same fragment length as the standard (Figure 4, lanes 2, 7-8). Of the three WWTP samples, all showed positive amplification; however, a faint additional band appeared slightly higher than the band of interest (Figure 4, lane 9, Figure 5, lanes 2-3).

Due to cross reactivity with one poultry sample, the HF183 genetic marker was tested against four additional poultry samples from 2 poultry houses on the same poultry farm in Somerset County, MD. Of the additional samples, all four showed positive amplification with the HF183 marker (Figure 6, lanes 6-9); however, poultry samples from this poultry farm are considered as one sample for specificity and sensitivity calculations. Positive poultry cross-reactivity with the HF183 primer set is consistent with multiple other analyses (Green et. al 2014, Shanks et. al 2010, Layton et. al 2013).

## **Gull**

Of the eighteen species, the Gull2 primers showed positive cross reactivity with two of the original poultry samples (Figure 8, lanes 8-9). The poultry samples that showed cross reactivity only contain fecal material, instead of poultry litter and fecal material combined. Poultry, but not poultry litter, cross reactivity was also observed in other studies (Ryu 2012). Sensitivity was tested using five gull samples from OCMD and two samples from Wicomico County Landfill (Figure 10). The Gull2 primers reacted strongly with four of the seven gull DNA extractions (Figure 10, lanes 3,5,6,8). There was also a faint band in one additional gull sample (Figure 10, lane 9). Of the gull samples that showed positive reactivity, three were from OCMD and one was from the Wicomico landfill.

## **Poultry**

Of the 18 original DNA extractions, the LA35 marker only amplified DNA from one sample: the poultry litter sample from Dover, DE (Figure 11, lane 3). Overall, the LA35 marker amplified two of the seven poultry samples, including the original chicken sample from Dover, DE and one of the four poultry samples from Somerset County (Figure 13, lane 6-7). Faint bands appeared in the three additional samples from the poultry farm in Somerset County as well (Figure 13, lane 3-5). We suspect that the faint bands are still positive results, as the same samples have showed strong amplification in a previous project (Dr. Mark Frana, communication). The faint bands could be the result of a low concentration of targeted DNA within the sample tube resulting in minimal amplification. Such results are not surprising, as this marker was originally described to detect poultry

litter with higher sensitivity than when the litter is not included in the original extraction sample (Weidhaas et. al 2010).

### **Ruminant**

The ruminant marker has previously reacted with cow and sheep fecal material. When tested against additional species, there were strong bands indicating cross reactivity with two raccoon samples, Canadian goose, two cow samples, and dairy goat. Faint bands appeared from multiple samples, including deer, chicken, rabbit, pigeon, and duck. The gel results and their analyses are not included.

### **Summary of Results**

A summary of the results for analyzed primers is presented in Table 2.

**Table 2.** Calculated specificity and sensitivity for studied genetic markers

	<b>Specificity</b>	<b>n value</b>	<b>Sensitivity</b>	<b>n value</b>
<b>Human, HF183</b>	89.5%	n= 18	100%	n=3
<b>Gull, Gull2</b>	70.5%	n=17	75%	n=8
<b>Poultry, LA35</b>	100%	n=15	50%	n=7

### **Discussion:**

Microbial source tracking is a useful way to identify the source of contamination in watersheds. It is important to identify the source of contamination, as fecal bacteria are potentially pathogenic depending on its host. This method allows for the remediation of watershed based on the level of human health risk (Boehm et. al 2013). Human fecal contamination is the most pathogenic so its identification has been deemed very

important. In order to accurately determine the main contributors of pollution in local watersheds, the specificity and sensitivity of primers must first be determined. Primers specifically designed to target suspect species should be studied in the region of interest prior to microbial source tracking analyses. This study measured the accuracy of three primer sets for use on the Eastern Shore of Maryland and on the coast of Delaware.

The human primers, HF183, originally described by Haugland et. al, showed cross reactivity with poultry litter samples from two different poultry houses. Such cross reactivity is of concern on the Eastern Shore due to the high prevalence of poultry houses on this region. The chance of obtaining false positive results with the HF183 primer set is of concern in further microbial source tracking studies on the Eastern Shore. The HF183 primer set should be tested against additional poultry and poultry litter samples from this region. The HF183 primer set was tested against three WWTP samples and showed positive amplification with all three samples. According to Ahmed et. al, there is no consensus on the number of samples needed to test the sensitivity of a genetic marker (2016). The concentration of the *Bacteroides* genus is higher in influent water samples compared to feces from one individual human (Ahmed et. al 2016). Although the sensitivity of the HF183 genetic markers was 100% in this region, additional WWTP samples should be tested.

The poultry genetic markers, LA35, showed positive amplification with poultry litter samples, which is consistent with the original description of this marker. Additional poultry and poultry litter samples should be tested against the LA35 marker in the future. Although the calculated sensitivity for the poultry primer set was only 50%, the specificity was 100%. Since cross reactivity was observed between the HF183 primer set

and poultry litter samples, influent WWTP samples should be tested against the LA35 genetic marker. Both genetic markers are from the *Bacteroides* genus, meaning there could be a close similarity in the 16S regions of the targeted *Bacteroides* species in humans and poultry.

The gull primer, Gull2, described by Lu et. al 2008, was 100% specific when tested against a wide array of avian and non-avian species. However, in a study by Sinigalliano et. al, the Gull2 primer set showed positive cross reactivity with pigeon samples (2013). This finding is consistent with the cross reactivity seen in this region. In addition to pigeon, cross reactivity was seen in all three original poultry samples and one cow sample. Sinigalliano et. al, detected low cross reactivity with such species as well (2013). In addition, a study by Ryu et. al, reported that the Gull2 genetic marker showed cross reactivity with 12.2% of chicken samples (2011). Comparatively, the specificity and sensitivity calculated in this study are slightly lower than previously reported Gull2 evaluations.

The Ruminant marker, RumBac2, will be further analyzed in order to comment on its specificity and sensitivity within this region. Although all ruminant species were detected by the primers, additional cross reactivity was seen. In order to enhance the results of the gels, the PCR conditions for the RumBc2 primers will be modified. Such modifications include altering the magnesium concentration, altering the annealing temperature to encourage more specific binding of the primers to the target sequence, and varying the concentration of primers in each PCR tube.

Due to low n values when calculating the specificity and sensitivity of each primer, additional samples could be tested. Confidently determining the source of

contamination in watershed along the Eastern Shore region is critical due to the high recreational use. All species targeted in this study have the potential to cause human disease. Human contamination is the most pressing, however, so identification of any human pollution is important. Since the HF183 primers cross react with poultry litter, further evaluation is necessary, in order to confidently report any concerns present.

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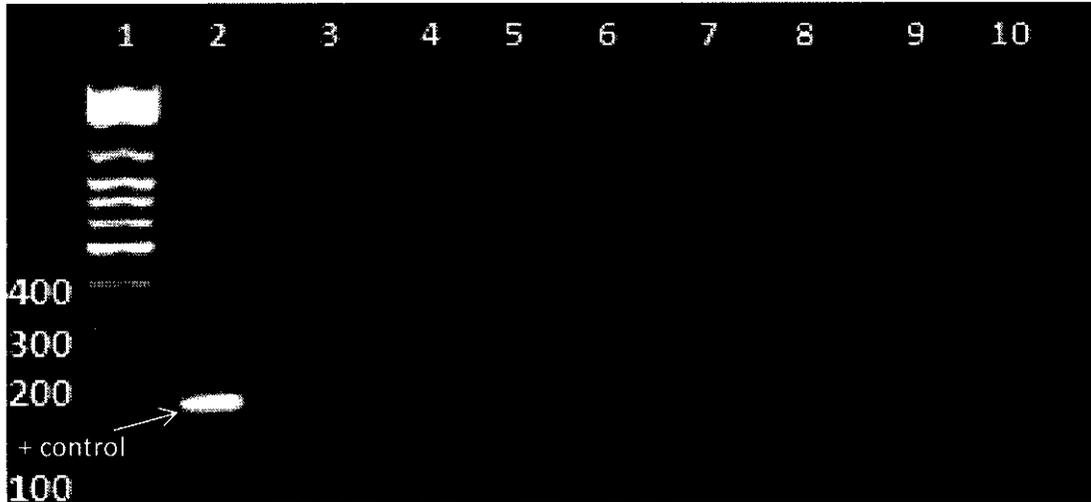


Figure 1. HF183 primers against samples 1-7. Lane1: DNA ladder, lane 2: HF183 standard, positive control, lane 3: Raccoon 1, lane 4: Raccoon 2, lane 5: Deer, lane 6: Canada goose, lane 7: Muskrat, lane 8: Cow 1, lane 9: Morning Dove, lane 10: Negative control (nuclease free water)

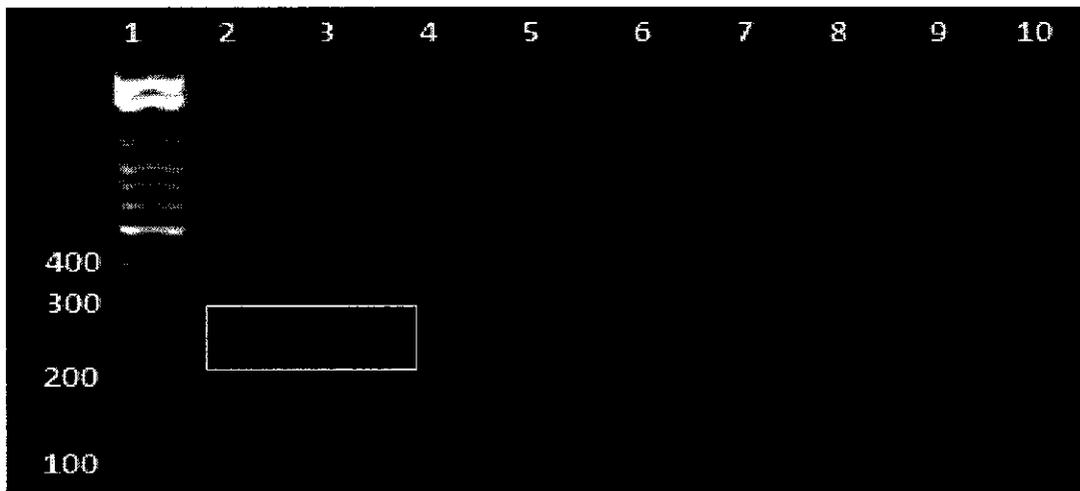


Figure 2. HF183 primers against samples 8 -15. Lane1: DNA ladder, lane 2: Dairy Goat, lane 3: Chicken 1, lane 4: Cow 2, lane 5: Gull, lane 6: Fox, lane 7: Chicken 2, lane 8: chicken 3, lane 9: Dog, lane 10: negative control (nuclease free water)

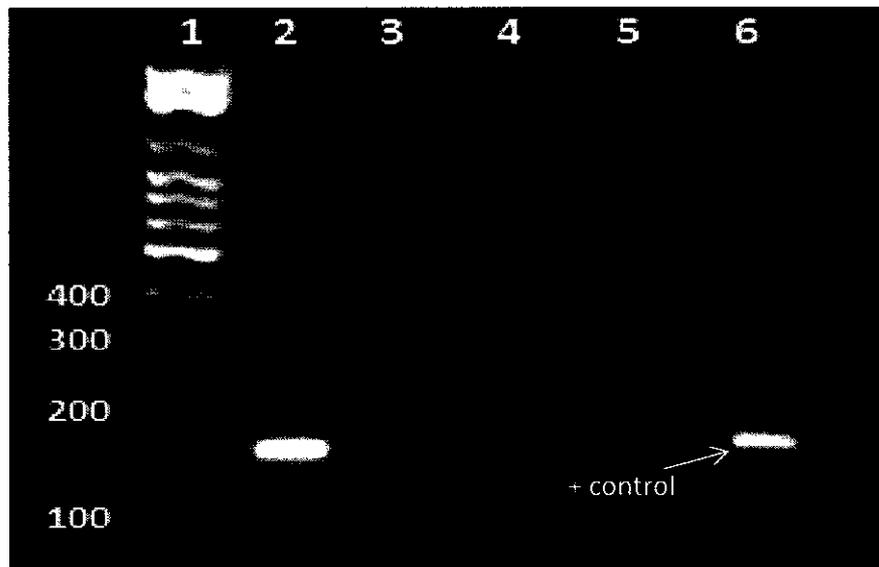


Figure 3. HF183 primers against samples 16 -18. Lane1: DNA ladder, lane 2: Rabbit, lane 3: Pigeon, lane 4: Duck, lane 5: negative control (nuclease free water), lane 6: HF183 standard, positive control

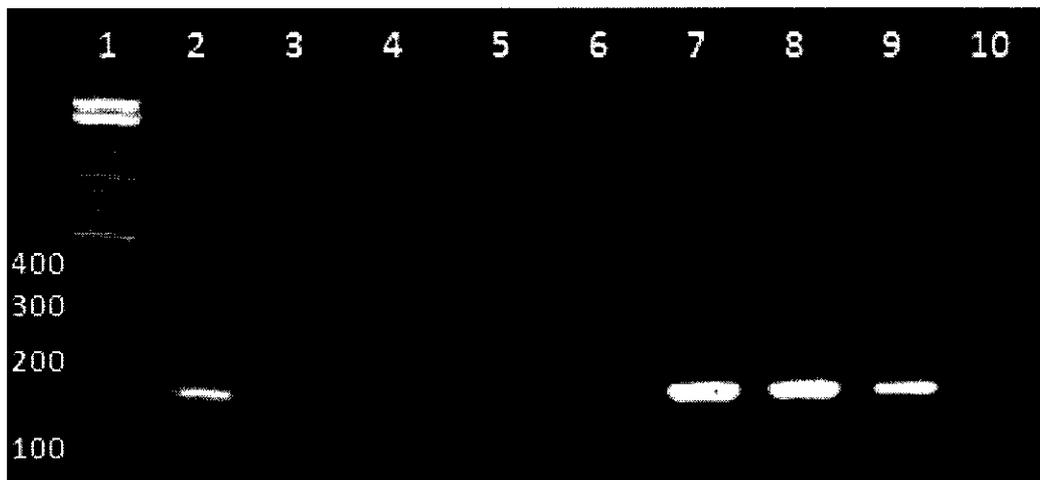


Figure 4. HF183 against reactive samples in duplicates. Lane 1: DNA ladder, lane 2: HF183 standard, positive control, lane 3: Rabbit, lane 4: Rabbit, lane 5: Dairy goat, lane 6: Dairy goat, lane 7: Chicken 1, lane 8: Chicken 1, lane 9: Lewes influent waste water treatment plant, lane: negative control (nuclease free water)

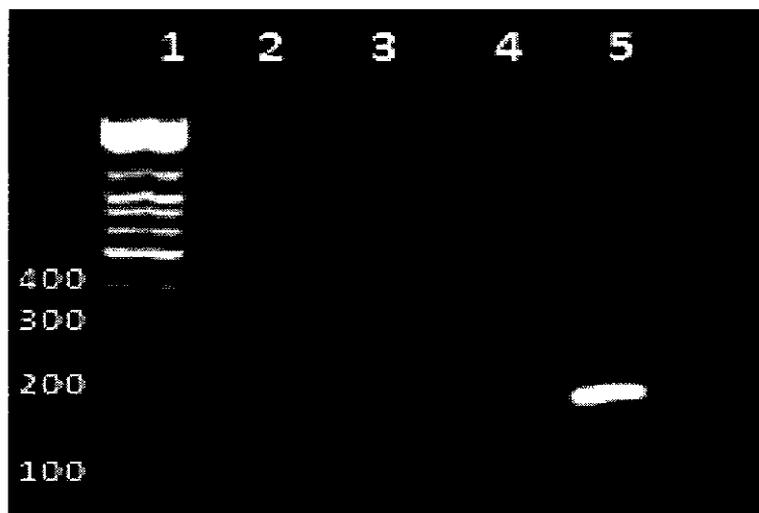


Figure 5. HF183 sensitivity continued. Lane1: DNA ladder, lane 2: Rehoboth influent WWTP sample, lane 3: Seaford influent WWTP sample, lane 4: negative control (nuclease free water), lane 6: HF183 standard, positive control

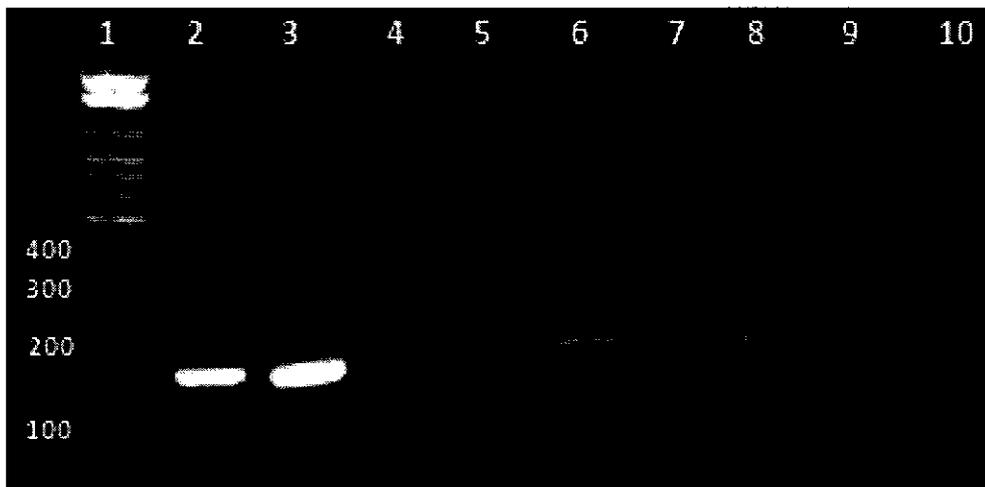


Figure 6. HF183 primers against poultry samples. Lane1: DNA ladder, lane 2: HF183 standard, positive control, lane 3: Chicken 1, lane 4: Chicken 2, lane 5: Chicken 3, lane 6: Somerset County poultry farm, house 5 (right), lane 7: Somerset County poultry farm, house 5 (left), lane 8: Somerset County poultry farm, house 6 (right), lane 9: Somerset County poultry farm, house 6 (left), lane 10: negative control, nuclease-free lane

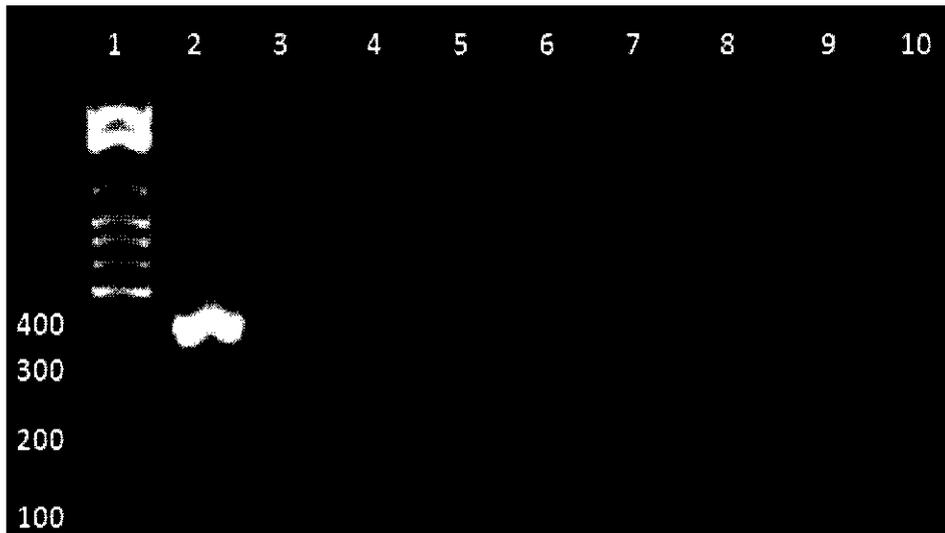


Figure 7. Gull2 primers against samples 1-7. Lane1: DNA ladder, lane 2: HF183 standard, positive control, lane 3: Raccoon 1, lane 4: Raccoon 2, lane 5: Deer, lane 6: Canada goose, lane 7: Muskrat, lane 8: Cow 1, lane 9: Morning Dove, lane 10: Negative control (nuclease free water)

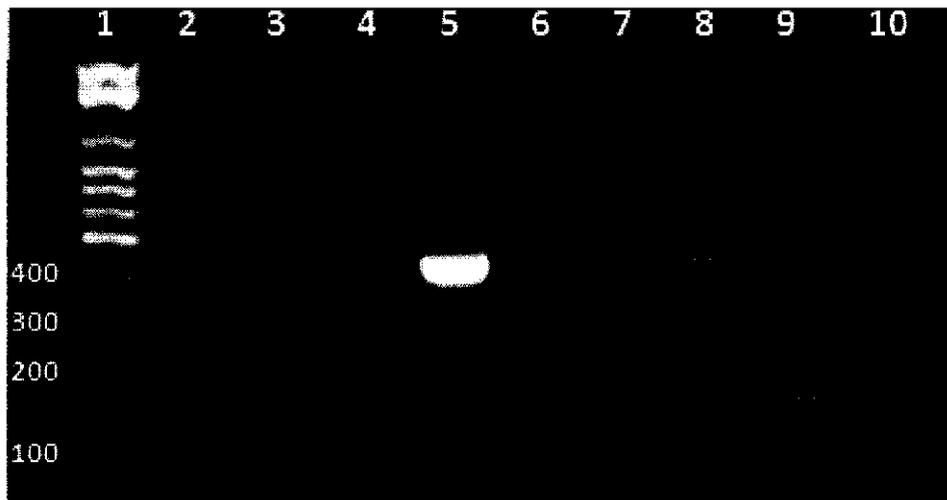


Figure 8. Gull2 primers against samples 8 -15. Lane1: DNA ladder, lane 2: Dairy Goat, lane 3: Chicken 1, lane 4: Cow 2, lane 5: Gull, lane 6: Fox, lane 7: Chicken 2, lane 8: chicken 3, lane 9: Dog, lane 10: negative control (nuclease free water)

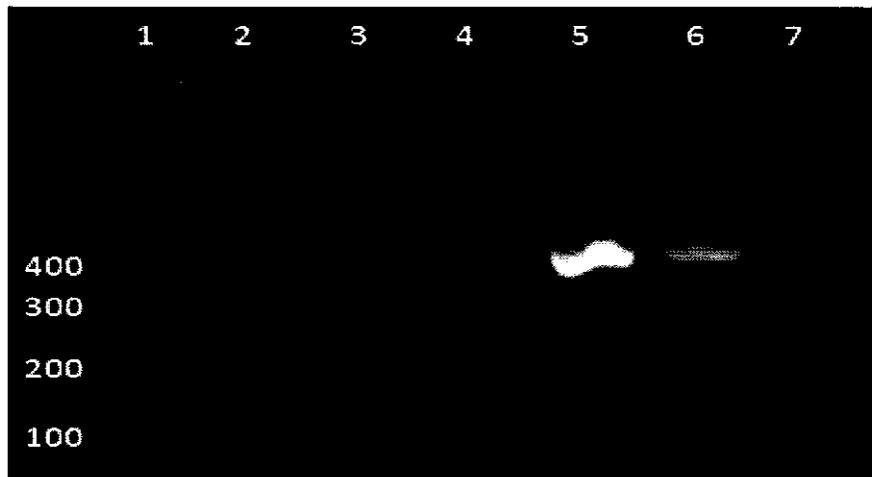


Figure 9. Gull2 primers against samples 16 -18. Lane1: DNA ladder, lane 2: Rabbit, lane 3: Pigeon, lane 4: Duck, lane 5: Gull samples from Wicomico County landfill sample 1, lane 6: Gull2 standard, positive control lane7: negative control (nuclease free water)

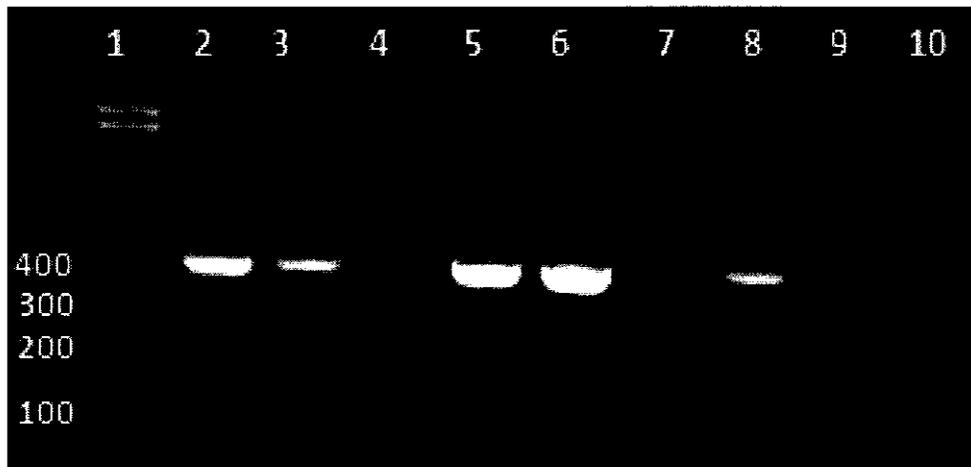


Figure 10. Gull2 sensitivity. Lane 1: DNA ladder, lane 2: Gull2 standard, positive control, lane 3: Ocean City, MD sample 1, lane 4: Ocean City, MD sample 2, lane 5: Ocean City, MD sample 3, lane 6: Ocean City, MD sample 4, lane 7: Ocean City, MD sample 5, lane 8: Wicomico County landfill sample 2, lane 9: Wicomico County landfill sample 3, lane 10: negative control (nuclease free water)

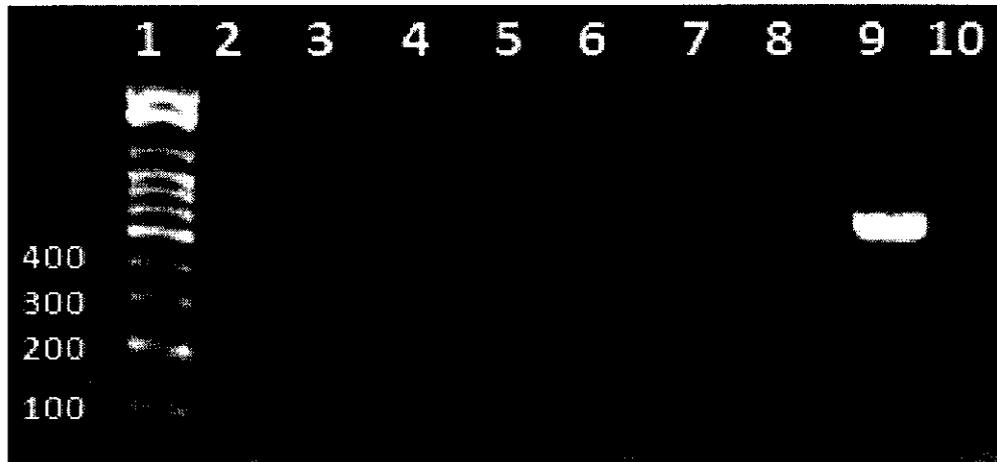


Figure 11. LA35 primers against samples 1-7. Lane1: DNA ladder, lane 2: Raccoon 1, lane 3: Raccoon 2, lane 4: Deer, lane 5: Canada goose, lane 6: Muskrat, lane 7: Cow 1, lane 8: Morning Dove, lane 9: LA35 standard, positive control, lane 10: Negative control (nuclease free water)



Figure 12. LA35 primers against samples 8 -15. Lane1: DNA ladder, lane 2: Dairy Goat, lane 3: Chicken 1, lane 4: Cow 2, lane 5: Gull, lane 6: Fox, lane 7: Chicken 2, lane 8: chicken 3, lane 9: Dog, lane 10: negative control (nuclease free water)

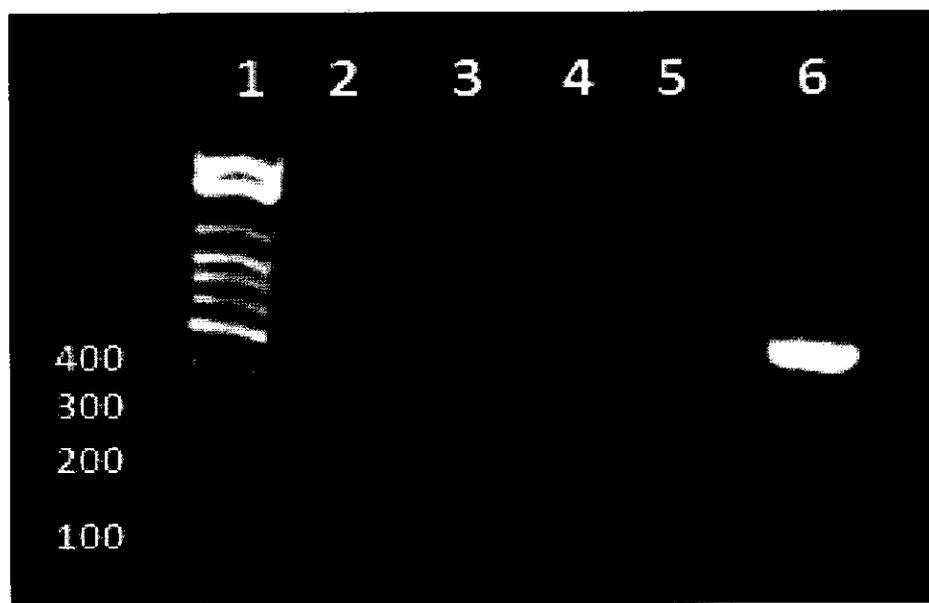


Figure 13. LA35 primers against samples 16 -18. Lane1: DNA ladder, lane 2: Rabbit, lane 3: Pigeon, lane 4: Duck, lane 5: negative control (nuclease free water), lane 6: LA35 standard, positive control

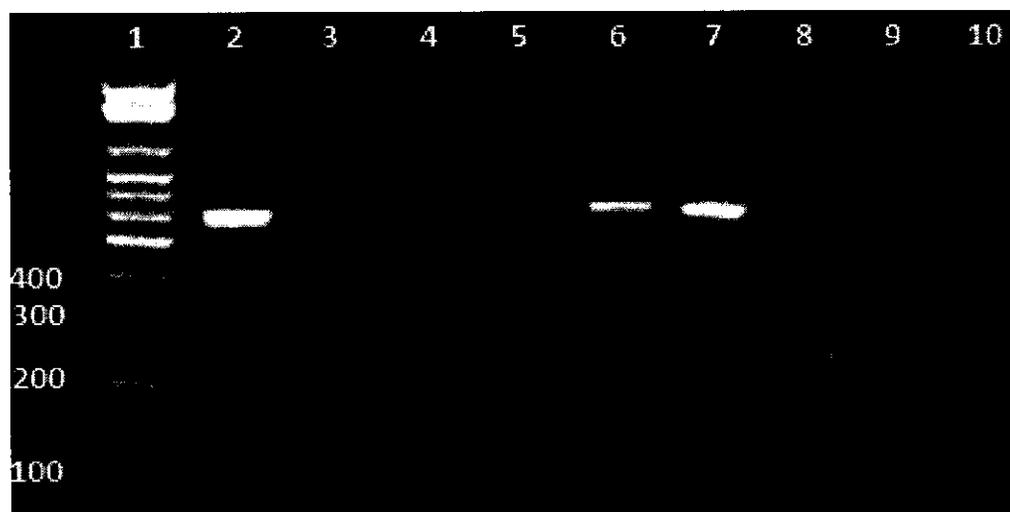


Figure 14. LA35 sensitivity. Lane1: DNA ladder, lane2: LA35 standard, positive control, lane3: Somerset County poultry farm, house 5 (right), lane 4: Somerset County poultry farm, house 5 (left), lane 5: Somerset County poultry farm, house 6 (right), lane 6: Somerset County poultry farm, house 6 (left), lane 7: chicken 1, lane 8: chicken 2, lane 9: Chicken 3, lane 10: negative control (nuclease free water)